Vertical Distribution of an Estuarine Snail Altered by a Parasite

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Estuarine snails *Ilyanassa obsoleta* bearing larvae of the trematode *Gynaecotyla adunca* behave singularly in comparison with conspecifics lacking this parasite. Following high tides, and especially at night, infected snails were found stranded high on beaches and sandbars. Semiterrestrial crustaceans living well up on the shore serve as the next host, and the modified (induced) snail behavior is apparently a parasite adaptation facilitating cercarial transmission to these crustaceans. The altered behavior is unusual because of its apparent enhancement of host-to-host transmission by cercariae rather than predation, the process commonly recognized as being enhanced by parasitic modification of host behavior.

THE ESTUARINE NEOGASTROPOD *Ilyanassa obsoleta* is an abundant, widespread organism in which parasites are of ecological significance (*1, 2*). I describe a pronounced effect of parasitism on *I. obsoleta* that serves to amplify and reemphasize the concept (*3, 4*) that knowledge of the ecological impact of parasites can be crucial. Larvae of the trematode *Gynaecotyla adunca* subordinate the normal behavior of this snail. During high water parasitized individuals crawl up onto beaches and sandbars leaving other conspecifics at lower levels. During summer, wherever this parasite is prevalent, vertical zonation of *I. obsoleta* populations is noticeably changed. Because there is no advantage to the host in this interaction, the parasite must be modifying host behavior to its own advantage.

The life cycle of *G. adunca* has been elucidated (*5*). Cercariae, produced in sporocysts within the snail, upon release encyst as metacercariae in certain species of semiterrestrial crustaceans. These include the beach hopper amphipods *Talorchestia longicornis* and *T. megalopthalmia*, and the fiddler crab *Uca pugilator*. Definitive hosts include a variety of shorebirds.

Studies were conducted at three sites in Delaware estuaries with semidiurnal tides. One site was 1.7 km of sandy beach along the south shore of Indian River Bay (75°09′W, 38°36′N) between Irons Lane Landing and Blackwater Beach. Snail distribution at this site was strikingly different from that at other sites (*1, 2*); numerous individuals were observed stranded on the beach during low water. To investigate this, five approximately equidistant stations were sampled eight times on daytime low tides between July 1983 and November 1984. At each station three sets of up to 25 snails were collected (as available) from 1 m or more (surface distance) above the waterline (AWL), the waterline plus or minus 1 m (WL), and 1 m or more below the waterline (BWL). Snails were dissected to determine sexual condition and occurrence of trematode parasitism (*2*).

The other two sites were 24 km to the north-northeast at Cape Henlopen (75°06′W, 38°47′N) just inside the mouth of Delaware Bay. In a beach study AWL and BWL snails were collected on 12 July 1984 along 250 m of beach and examined. A sandbar study (Fig. 1) was conducted between 21 July and 5 August (*6*). A plot (3 by 5 m) was marked near the sandbar peak. Vertical elevations were determined every 3 m along eight transects originating at plot center and used to generate a contour map (Fig. 1). On 29 occasions for 30 consecutive low tides the sandbar plot was inspected, and snails within were collected and counted. Among 1929 snails present 271 were examined in the laboratory. Others were ejected. Therefore, on a given low tide inhabitants had entered the plot during the preceding high water (~6 hours). For comparison 30 peripheral samples of ten snails were collected during the study from the sandbar and adjacent sandflat (Fig. 1). All 300 snails were examined in the laboratory. Contingency analyses (*7*) were used to compare frequencies.

Parasitism frequencies in Indian River Bay snails are grouped according to shoreline positions in Fig. 2. Since sex has been shown to influence *I. obsoleta* behavior (*2*), I confirmed for uninfected snails that sex and vertical position were independent (*8*). However, there is a strong relation between vertical position and parasitism (Fig. 2): snails infected with *G. adunca*, whether singly or multiply, were more frequently AWL and less frequently BWL than expected. Conversely, unparasitized snails and those parasitized with *Lepocreadium setiferoides* or *Zoogonus rubellus* were more frequently BWL and less frequently AWL.

In the Henlopen beach study ratios of snails with and without *G. adunca* were AWL, 22:3; BWL, 0:25. This shows an association of high intertidal snail position with *G. adunca* presence [$\chi^2(1) = 39.286$, $P < 0.001$]. The Henlopen sandbar pattern (Fig. 3) was similar to that on both beaches. Snails infected with *G. adunca*, either singly or doubly, were inside the plot (Fig. 1) more frequently and outside the plot less frequently than expected. Unparasitized snails and *Z. rubellus*–bearing snails showed the reverse. Sex was not a statistically significant factor (*9*).

The strong association between *G. adunca* presence and altered host zonation at all sites indicates a cause and effect relation wherein *G. adunca* alters normal host behavior. Counts of snails invading the plot during high tides reveal time needed to generate the pattern and that the phenomenon has a diurnal aspect. Overall incidence of *G. adunca* in plot snails was 86.3%. This incidence (range, 75 to 100%, $n = 8$) was characteristic of plot snails whether collected day or night, so the observed pattern is produced with each tide. However, the pattern varies diurnally: more *G. adunca*–bearing snails occupied the plot during nighttime low tides (mean ± SD, 117.8 ± 43.8; range, 66 to 194; $n = 12$) as com-

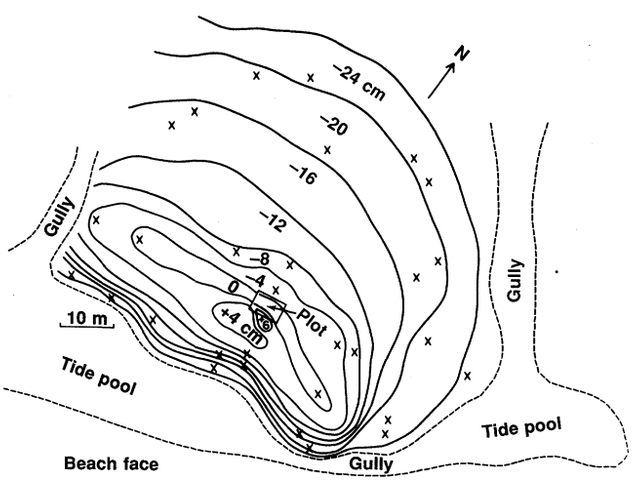

Fig. 1. Elevation zones on the Cape Henlopen sandbar used to study trematode parasitism and *Ilyanassa obsoleta* vertical distribution. Position of the experimental plot is shown and locations of peripheral snail samples are indicated by Xs.

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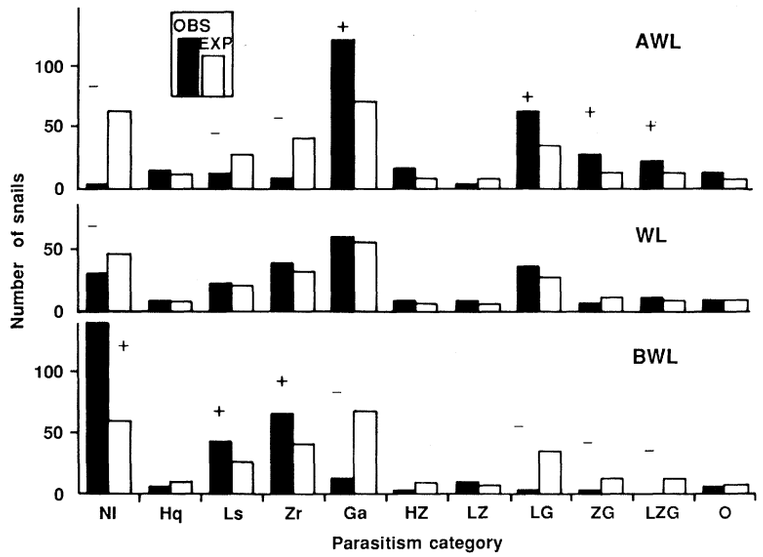

Fig. 2. Frequencies of *Ilyanassa obsoleta* trematode parasitism according to position on shore in Indian River Bay, Delaware. A 3 by 11 contingency analysis showed that parasitism and vertical position were not independent [$\chi^2(20) = 441.6$, $P < 0.001$]. Categories that would give a statistically significant χ^2 ($P < 0.01$) in a test with 1 degree of freedom are marked with a plus if observed (OBS) frequency is greater than expected (EXP) and with a minus if less. Single infections: NI, not infected; Hq, *Himasthla quissetensis*; Ls, *Lepocreadium setiferoides*; Zr, *Zoogonus rubellus*; and Ga, *Gynaecotyla adunca*. Multiple infections are abbreviated with genus initials of species involved. Categories with expected frequencies less than 5 were grouped into O (other) which includes: *Austrobilharzia variglandis* (Av); *Stephanostonum dentatum* (Sd); *S. tenue* (St); HG; LA; ZSd; ZD (D, *Diplostonum nassa*); AG, GSt; GD, ZAG; ZGSd; and LAG.

pared with daytime low tides (mean ± SD = 30.4 ± 20.4; range, 4 to 64; n = 17).

Chemically mediated parasitism effects in *I. obsoleta* are known (*10*), and *G. adunca* may induce behavior similarly. If so, the isolated molecule or molecules could be useful in controlling snails that harbor medically significant parasites (for example, schistosomes) if host behavior can be altered predictably. The dominance of *G. adunca* in multiple infections (Figs. 2 and 3) lends support to this proposition.

Since parasitized snails are virtually all sterile (*1*) any adaptive value in altered host behavior goes to the parasite. The most attractive explanation is that the observed behavior is a parasite adaptation facilitating life-cycle completion. The environmental challenge seems to be getting cercariae from an aquatic first host to a semiterrestrial second host. Similar parasite adaptations usually involve altering intermediate host behavior to make predation by a definitive host more likely (*3*, p. 150). The adaptation of *G. adunca* is unusual, however, because it enhances transmission not to a definitive host by predation, but to a second intermediate host by cercariae. Precisely how transmission is accomplished is not known. In the study areas the second host is probably a beach hopper. Presumably cercariae, released from snails as they travel the beach during high water, infest beach hoppers when they invade the beach during low water.

The day-night difference in numbers of snails in the plot (*11*) could result from equal invasions with more snails remaining at night or unequal invasions. There were no mass emigrations of plot snails at exposure on daytime low tides, which supports the differential migration proposition. From an adaptive standpoint, the diurnal pattern could be a strategy to avoid daytime exposure (*12*) or to take advantage of a behavior pattern in the second host such that cercarial transmission is more probable at night, or both.

Gynaecotyla adunca occurred in multiple infections with all observed trematodes at both study sites. Among Indian River Bay snails 27.6% were multiply infected and 78.4% of these had *G. adunca*. Corresponding Henlopen sandbar figures (35.2 and 88.6%) are similar. These proportions of multiple infections in *I. obsoleta* are larger than reported in previous studies. In Rhode Island, 0.9% double infections were found among 5,713 snails (*13*). Two studies in North Carolina revealed 0.02% multiple infections among 5,025 snails (*14*), and none among 14,978 snails (*15*). In a previous Henlopen study (*2*), I found 9% multiple infections among 2,111 snails. *Gynaecotyla adunca* was infrequent or absent in these studies, and the positive relation between *G. adunca* and multiple infection frequencies seems clear.

Trematode species interact in various ways in multiple infections (*2, 16*), and a diversity of effects on hosts and other parasites can be expected. Presumably each parasite will influence the host to its own advantage. With *I. obsoleta* vertical distribution it is clear that *G. adunca* influence dominates (Figs. 2 and 3). However, in other contexts [for example, carrion response (*2*)] outcomes of contrasting parasite influences can be intermediate. Intermediate hosts may thus be more variously and extensively influenced by parasites than is generally realized.

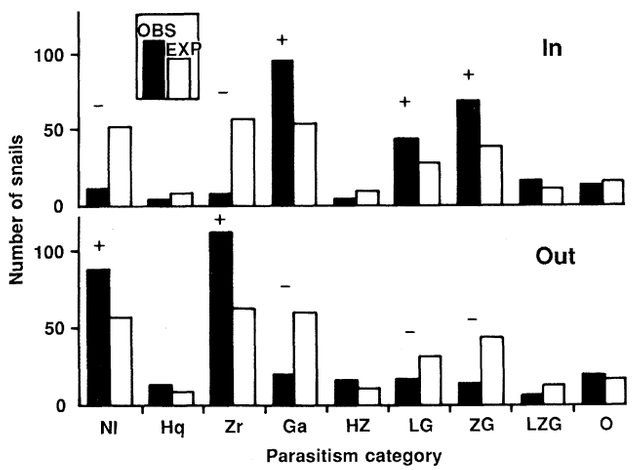

Fig. 3. Frequencies of *Ilyanassa obsoleta* trematode parasitism in and out of experimental plot on the Cape Henlopen sandbar (Fig. 1). A 2 by 9 contingency analysis showed that distribution was not independent of parasitism [$\chi^2(8) = 252.2$, $P < 0.001$)]. Abbreviations and protocol are described in the legend to Fig. 2. Other (O) category includes: Ls; Av; HG; LZ; AG; HZG; and LAG.

REFERENCES AND NOTES

1. L. A. Curtis and L. E. Hurd, *Ecology* **64**, 819 (1983).
2. L. A. Curtis, *Biol. Bull.* **169**, 377 (1985).
3. P. Price, *Evolutionary Biology of Parasites* (Princeton Univ. Press, Princeton, NJ, 1980).
4. M. Rothschild, *Nature (London)* **193**, 1312 (1962).
5. J. S. Rankin, *Biol. Bull.* **79**, 439 (1940); W. S. Hunter, *J. Parasitol.* **38**, 308 (1952); W. S. Hunter and W. B. Vernberg, *ibid.* **43**, 493 (1957).
6. To locate Henlopen sample sites, see sample numbers in Curtis and Hurd (*1*, figure 1, beach, 34–36; sandbar, 23).
7. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1981).
8. Female-to-male ratios were WL, 18:12; BWL, 77:63; $\chi^2(1) = 0.251$; $P > 0.05$.
9. Female-to-male (uninfected only) ratios were plot, 14:8; peripheral, 40:48; $\chi^2(1) = 2.328$; $P > 0.05$.

10. E. J. Pearson and T. C. Cheng, *J. Invert. Pathol.* **46**, 239 (1985).
11. Snails could have been present during the day but buried. On three daytime tides after evicting snails I scratched 4 to 5 cm into the entire plot. A few snails were unearthed but never enough to approach nighttime counts. Moreover, snails tend to unbury when submerged by tides. Never was there a marked unburying after daytime low tides.
12. C. W. Schaefer, P. W. Milch, N. L. Levin, *Nautilus* **81**, 109 (1968). Mean daytime temperature of the plot surface sand was 25.4°C (SD, 2.7; $n = 9$). The snail can tolerate this, and if either party is at risk, it is probably the parasite.
13. J. J. Gambino, *J. Parasitol.* **45**, 440, 456 (1959).
14. W. B. Vernberg, F. J. Vernberg, F. W. Beckerdite, *Science* **164**, 1287 (1969).
15. J. S. McDaniel and J. R. Coggins, *J. Elishia Mitchell Sci. Soc.* **88**, 55 (1972).
16. P. J. DeCoursey and W. B. Vernberg, in *Symbiosis in the Sea*, W. B. Vernberg, Ed. (Univ. of South Carolina Press, Columbia, 1974), pp. 93–109.
17. My thanks to M. R. Carriker and L. E. Hurd for valuable comments on the manuscript and to A. M. Barse for field assistance. Partial funding was provided by a Biomedical Research Support Grant from the University of Delaware.

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F-Actin and Microtubule Suspensions as Indeterminate Fluids

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The viscosity of F-actin and microtubule suspensions has been measured as a function of shear rate with a Weissenberg rheogoniometer. At shear rates of less than 1.0 per second the viscosity of suspensions of these two structural proteins is inversely proportional to shear rate. These results are consistent with previous in vivo measurements of the viscosity of cytoplasm. This power law implies that shear stress is independent of shear rate; that is, shear stress is a constant at all shear rates less than 1.0 per second. Thus the flow profile of these fluids is indeterminate, or nearly so. This flow property may explain several aspects of intracellular motility in living cells. Possible explanations for this flow property are based on a recent model for semidilute suspensions of rigid rods or a classical friction model for liquid crystals.

ACTIN AND MICROTUBULES ARE among the most widely conserved structural proteins within eukaryotic cells. It has long been suspected that the viscoelastic properties of these polymers (particularly F-actin) play an important role in the maintenance and development of cell shape and in cellular motility (*1*). Maruyama *et al.* (*2*) found that the measured viscosity of F-actin was precisely inversely proportional to the shear rate. These workers reported on the uniqueness of this inverse proportionality and speculated that it may be the result of the formation of an actin network. Subsequent rheological studies confirmed that F-actin suspensions are shear thinning but did not find the same shear-viscosity dependence or did not discuss this relationship (*3, 4*). We confirm the observation of Maruyama *et al.* for actin and observe a similar inverse proportionality for suspensions of microtubules. Also, we show that as a result of this dependence, the fluid flow profiles for such suspensions are indeterminate. That is, a given force does not fix the velocity of movement in these fluids.

Figure 1 is a logarithmic plot of viscosity versus shear rate for two runs of actin at different concentrations, two runs of microtubules with and without taxol [a stimulator of microtubule assembly (*5*)], and one run of the viscosity standard. The values reported in Fig. 1 and throughout this report are the stable values obtained after shearing the fluids for several seconds. As found previously for actin (*2*) in both actin and microtubule suspensions, the torque rises sharply as shear begins but then declines to a stable value over a time period that depends on the shear rate. In addition to the shear-thinning behavior characteristic of all polymer suspensions, these plots show that the power law dependency for actin and microtubules is nearly -1 for all concentrations. That is, actin and microtubule suspensions behave as power law fluids with

$$\eta = A\dot{\gamma}^n \qquad (1)$$

where η is viscosity, A is a constant, $\dot{\gamma}$ is shear rate, and n is the power law exponent. In 12 runs of actin at concentrations from 2 to 6 mg/ml the exponent varied from -0.85 ± 0.02 ($\pm$ standard error of regression coefficient) to -1.15 ± 0.01 with a mean of -1.00. Both extreme values occurred for actin at 2 mg/ml with the rheogoniometer at its lower limit of resolution. In seven microtubule runs the exponent varied from -0.90 ± 0.01 to -1.03 ± 0.03 with a mean of -0.94. The -1 slope (on log-log plots) was found to be nearly independent of the shearing history of the sample, as shown in Fig. 2. The slope of the line for increasing shear (-1.00 ± 0.02) is nearly identical to that for decreasing shear (-1.01 ± 0.02). In a run of chromatographed actin (3 mg/ml) in which shear was varied at random the power law dependence was -0.99 ± 0.09. The power law dependence of the microtubule suspension viscosity was similarly insensitive to shear history. Our measurements varied only slightly. The small standard errors of regression coefficients indicate little variation of points around the calculated line. The slope from run to run also varied little at nominally the same conditions. In five runs of actin at 6 mg/ml from three different actin preparations the mean slope was 1.01 ± 0.04 (SEM). The possibility that our results are due to slipping at rheometer surfaces is eliminated by the finding that actin and microtubule suspensions pour easily and are well mixed after the shearing process in the rheometer. The observed viscosities for suspensions of these two filamentous constituents of the cytoplasm are consistent with previous in vivo measurements of cytoplasmic viscosity. Figure 3 shows a comparison of data obtained from various cytoplasmic studies with that of F-actin suspension at 3 mg/ml. This similarity of the viscosity function in vitro and in vivo is particularly interesting in view of the lack of true cross-links within purified F-actin (*6*) and the highly cross-linked structure of cytoplasm (*7*).

Power law observations in this range are significant because they contradict Graessley's classic random-coil-entanglement explanation that suggests the minimum n value is $-9/11$ (*8*). Typically, data show n between -0.4 and -0.85 for polymer solutions (*9*). Significantly, since shear stress is viscosity multiplied by shear rate

$$\tau = \eta\dot{\gamma} = (A\dot{\gamma}^n)\dot{\gamma} = A\dot{\gamma}^{(n+1)} \qquad (2)$$

where τ is the shear stress (force per unit area). Our observation that $n = -1$ means that the shear stress is independent of shear rate. In this case, fluid motion is completely indeterminate. In Fig. 4 we plot shear stress data for actin, microtubules, and the Newtonian fluid standard. One's intuition, for example, on the basis of flooded rivers, is that increased shear increases shear stress. As shown in Fig. 4, the Newtonian standard

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